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The sweet smell of success

Smell is arguably the most evocative and mysterious of our senses. But thanks to advances in our understanding of the cells that detect odour, its secrets should now start to be revealed. Carina Dennis sniffs around.

Lawrence Katz likes to start his lectures to medical students by opening a vial containing the pig version of the pheromone androstenone, used by boars to attract their mates. Katz, a neuroscientist at Duke University Medical Center in Durham, North Carolina, can't smell it himself. But many of his students retch in their seats at the pungent odour. "My graduate student says it's like having his head shoved in a urinal," says Katz.

Such individual differences in sensitivity to smells are thought to be the result of variations between olfactory receptors — proteins carried on the surface of sensory neurons that detect volatile chemicals wafting up our nasal passages. We have hundreds of distinct receptors, which between them can distinguish thousands of different chemicals. But without a clear understanding of how this information is encoded by olfactory receptors, scientists have made little progress in determining how our brain perceives complex fragrances such as 'chocolate' or 'freshly baked bread'.

"The mammalian nose is the best chemical detector in the world, yet we still don't understand how it codes," says Stuart Firestein, a neuroscientist at Columbia University in New York, whose team was the first to document the molecular interaction between a specific receptor and a particular odorant[1].

That should now start to change. Experiments are beginning to reveal how individual sensory cells end up carrying just one type of receptor. Researchers should also soon be able to culture cells featuring any receptor they want. This will make it easier to work out which receptors respond to which odours. And that, in turn, should help scientists to track the neural processing of smell, all the way from sensory cells to the brain's cortex.

Odour eaters

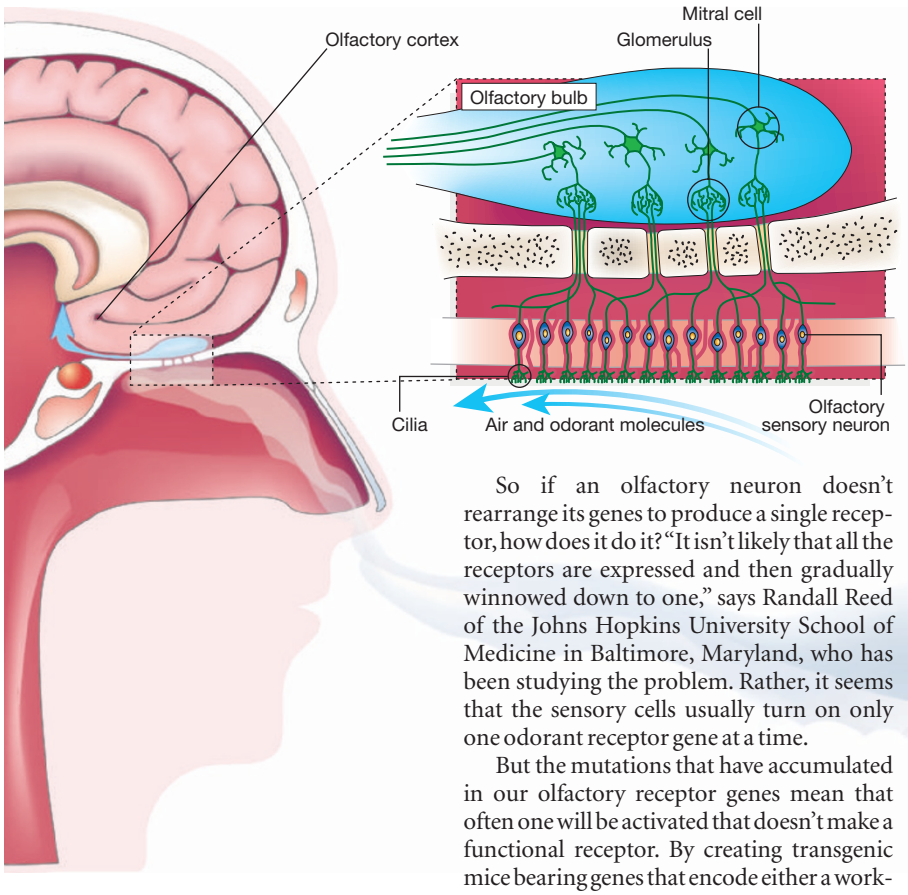
Our sense of smell begins with two tiny patches of tissue that line the roof of our nasal passages, just below eye level (see Figure, opposite). Crammed into these patches, which together are about as big as a postage stamp, are the ends of some 10 million sensory neurons. Minuscule hair-like extensions, called cilia, project from these cells and carry the olfactory receptors.

These receptors are encoded by the largest family of genes known to exist in mammals. Mice, which rely heavily on their sense of smell, have about 1,200 of these genes, most of which are in full working order[2]. By contrast, nearly two-thirds of the roughly 1,000 human olfactory receptor genes, which are spread over all but two of our chromosomes, have accumulated mutations that render them useless[3]. It seems that we have been losing functional olfactory receptors ever since our primate ancestors evolved full colour vision[4].

But even this diminished sense of smell is fiendishly difficult to investigate compared with vision. It is relatively easy to determine what an individual sensory cell in the retina responds to, in terms of wavelengths of light and a defined area within the field of vision. Over the past few decades, neuroscientists have used electrophysiological recordings to work out how responses are combined and processed to perceive more complex visual patterns.

Olfactory scientists have not been able to build from such firm foundations. A single odorant chemical can be recognized by multiple olfactory receptors, and each recep-

Follow your nose: how we detect odours



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tor is thought to recognize several odorants. Individual cells carry only one type of receptor, which means that the detection of a particular odorant is encoded by the firing of a distinct combination of sensory cells. But that code remains unbroken: smell researchers have identified the volatile odorant partners of only about a dozen mammalian olfactory receptors.

One piece at a time

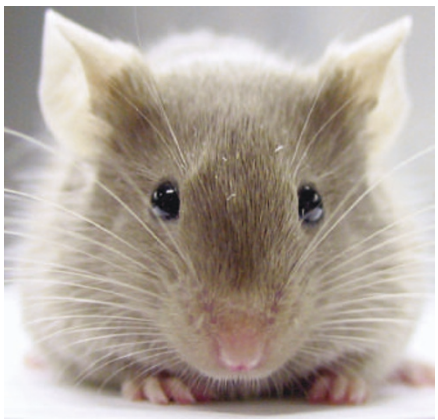
More fundamentally, researchers haven't been able to work out how each sensory neuron comes to bear just one type of receptor. One idea was that the mechanism might involve an irreversible rearrangement of the DNA of olfactory receptor genes, in much the same way as the B cells of our immune system cut and paste their genes to produce just one particular antibody.

But there was little supporting evidence, and cloning technology has now disproved this theory. If you clone a mouse from a single B cell, its entire immune system can generate only one antibody[5]. Two groups have repeated the same experiment with mature olfactory neurons, and shown that the resulting mice have the normal diversity of olfactory receptors[6,7]. "Cloning was a straightforward way to test the question," says Peter Mombaerts of Rockefeller University in New York, whose team reports its results in this issue of *Nature*[7].

So if an olfactory neuron doesn't rearrange its genes to produce a single receptor, how does it do it? "It isn't likely that all the receptors are expressed and then gradually winnowed down to one," says Randall Reed of the Johns Hopkins University School of Medicine in Baltimore, Maryland, who has been studying the problem. Rather, it seems that the sensory cells usually turn on only one odorant receptor gene at a time.

But the mutations that have accumulated in our olfactory receptor genes mean that often one will be activated that doesn't make a functional receptor. By creating transgenic mice bearing genes that encode either a working or a non-functional receptor, teams led by Reed and by Hitoshi Sakano at the University of Tokyo have independently shown that once a sensory neuron hits upon a gene that encodes a working receptor, no others are activated[8,9]. The cell's fate then seems to become fixed, and it carries on producing the same receptor for the rest of its life[7].

But how the cells ensure that multiple genes are not turned on at once remains unclear. Perhaps the genes must interact at random with a molecule that is only available in very limited quantities, such as a factor needed to free DNA from its dense packing within a chromosome. Sakano has another theory. He suspects that olfactory receptor genes need to interact with a stretch of DNA, known as a locus control region (LCR), located some distance away on the same chromosome. This must loop around and snuggle up against the olfactory genes, Sakano argues, but can only do so with one gene at a time. For one cluster of olfactory receptor genes, he has already identified a candidate LCR, which he calls the 'H' region. When it is deleted, the genes in the cluster remain inactive[8].



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Smelly beast: this mouse, cloned from an olfactory neuron, has a normal sense of smell.

Knowing how individual sensory cells come to produce one particular receptor will still leave smell researchers in the dark about how the cells collectively encode odours. To help crack this code, scientists want to study the interaction between receptors and different odorants for individual cells. But olfactory sensory cells are hard to culture and handle in the lab. "They are difficult to grow and nearly impossible to separate from their neighbours," says Mombaerts. That poses a big problem for researchers trying to crack the olfactory code, as neighbouring cells bear different receptors.

Follow the scent

An alternative would be to genetically engineer cells that are more amenable to culturing in the lab to make them carry particular olfactory receptors on their surfaces. But attempts to do this have been frustrated by the failure of the olfactory receptors to transfer to the outer membrane of the engineered cells.

Hiroaki Matsunami at Duke University may now have removed this experimental bottleneck. In unpublished work, he has figured out that olfactory receptors need a little help from a specific protein to find their way to the cell's surface. If the receptor genes are introduced into cells together with the gene encoding this protein, they can find their way to the exterior. Matsunami is beginning to use these 'hana' cells — the name derives from the Japanese for nose — to identify which odorant molecules are recognized by different receptors.

That won't be a simple task. New evidence from a team led by Kazushige Touhara at the University of Tokyo's Graduate School of Frontier Sciences in Chiba suggests that some odours can also inhibit olfactory receptors, rather than activate them[10]. This means that our nasal cavities represent a competitive battleground where odours jockey with each other to stimulate or block olfactory receptors. This concept has been recognized by perfume companies, which add molecules to their products to enhance a fragrance.

As more odours are added to a mixture, some will cancel others out or change how we perceive the mixture's smell. "We have a physiological limit of about three in the number of odours we can distinguish at once," says David Laing, a neuroscientist at

the University of New South Wales in Sydney, Australia[11]. For instance, if you were given a vial with equal concentrations of banana, vanilla and strawberry smells, you will be able to distinguish all three odours. But throw in peppermint and chances are you won't be able to discriminate between all four. And the combination of odours may smell completely different from any of the individual components. "We can't predict what we are going to smell with various combinations of odours," says Laing.

But if smell researchers do crack the olfactory receptor code, neuroscientists interested in the sense of smell will find themselves in the position that their colleagues who study vision have enjoyed for several decades. They will be able to study various cells within the system, and relate the coding of information at the level of the receptors to higher levels of processing.

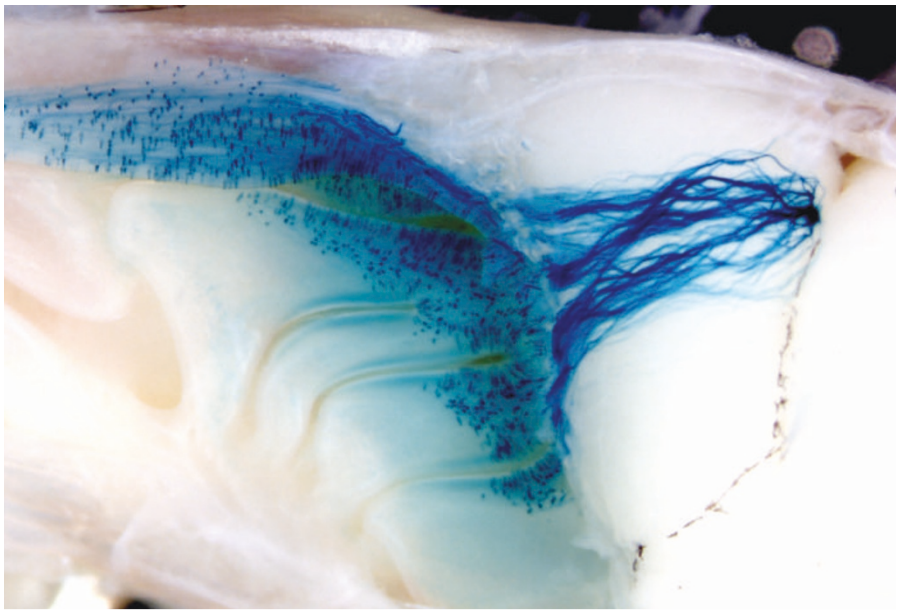
Smells like team spirit

The basic wiring of the olfactory system is already known. Sensory neurons send projections back to the olfactory bulbs, a pair of pea-sized structures nestled on the underside of the frontal lobe of the brain, set back behind our eyes. Each bulb is divided into two halves, and each of these halves contains hundreds of structures called glomeruli. These act like neural junction boxes linking the projections from sensory cells to neurons called mitral cells that relay information about smell to olfactory centres in the brain.

Sensory cells bearing the same receptor all converge in the same glomeruli, and each half of each olfactory bulb contains at least one glomerulus for each type of receptor. As a result, an odour that stimulates a specific group of sensory neurons will also cause particular glomeruli to fire — odours can be thought of as being coded through a 'spatial map' involving the firing of glomeruli at specific positions in the olfactory bulbs.

From here, information is relayed back to olfactory centres of the brain's cortex, which interpret the odours and direct our responses to them. But opinions differ on the extent to which this processing begins in the olfactory bulbs themselves.

Two research groups conducted different experiments to measure the input and output of the insect equivalent of the mammalian olfactory bulb, called the antennal lobe, and arrived at very different conclusions. From their physiological recordings, Richard Axel of Columbia University and his colleagues argue that the spatial map encoded by the antennal lobe is faithfully relayed by the neurons leaving the structure[12]. But Gilles Laurent of the California Institute of Technology in Pasadena has evidence that the output of the antennal lobe looks very different from the input, with the information spread across a much greater number of outgoing neurons[13,14]. He believes that not only do the outgoing neurons tell the brain which receptors were activated, but that they also provide information about an odour through the timing of the signals they transmit[15].



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Nasal passage: smells are picked up by receptors on cilia (left, dark orange) at the end of a sensory neuron (light orange). Sensory cells bearing the same receptor (stained blue, above) then connect to the same glomerulus (top right).

Whatever the case, it is unclear whether what happens in insects also applies to mammals. "The insect antennal lobe is thought to be quite similar to the mammalian olfactory bulb, but that still remains to be shown," says Laurent.

Higher levels of processing in the brain's olfactory cortex remain, for now, obscure. Some researchers argue that the neural connections to the cortex are so diffuse that it is impossible to discern any spatial map of odour coding. But Linda Buck of the Fred Hutchinson Cancer Research Center in Seattle, Washington, has used genetic tricks to track the flow of information to the cortex in response to the activation of particular olfactory receptors. Her findings suggest that input from sensory neurons bearing a particular olfactory receptor is relayed to discrete clusters of neurons in the cortex[16].

But the resulting spatial map is different from that in the olfactory bulbs. For a start, it seems that olfactory neurons bearing a particular receptor can cause activity in several distinct regions of the cortex. "There is a real transformation of information in the olfactory cortex — and it's very complex," says Buck.

That complexity is likely to involve emotional responses and information from our memories. Olfactory scientists still have much work to do before they understand exactly how the experience of a particular odour can have the dramatic effect of Katz's boar pheromone. But thanks to the advances that are now being made, it is at least starting to look like a tractable problem. ■

Carina Dennis is *Nature*'s Australasian correspondent.

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